Human MSWLLFLAHRVALAALPCRRGSRGFGMFYAVRRGRKTGVFLTWNECRAQVDRFPAARFKKFATEDEAWAFVRKSAS
Chicken MLRWL-----VAL-LSHSCFVSKGGGMFYAVRKGRQTGVYRTWAECQQQVNRFPSASFKKFATEKEAWAFVGAGPP
Yeast MARQGNFYAVRKGRETGIYNTWNECKNQVDGYGGAIYKKFNSYEQAKSFLGQPNT
Mouse EST GICGLGMFYAVRRGRRPGVFLSWSECKAQVDRFPAARFKKFATEDEAWAFVRSSSS
Human PEVSEGHENQHGQESEAKXXKRLREPLDGDG----------
Chicken DGQQSAPAETHGASAVAQENASHREEPETDV----------
Yeast TSNYGSSTHAGGQVSKPHTTQKRVHRRNRPLHYSSLTSSSACSSLSSANTNTFYSVKSNVPNIESKIFNNWKDCQA
Mouse EST PDGSKGQESAHEQKSQAKTSKRPREPL
Human ----------HESAXPYAKHMKPSVEP-APPVSRDTFSYMGDFVVVYTDGCCSSNGRRXPRA
Chicken ---------LCCNACKRPYEQSTNEEHTVRRAKH---DEEQSTPVVSEAKFSYMGEFAVVYTDGCCSGNGRNRARA
Yeast YVKHKRGITFKKFEDQLAAENFISGMSAHDY-KLMNISKESFESKYKLSSNTMYNKSMNVYCDGSSFGNGTSSSRA
E.coli MLKQVEIFTDGSCLGNPGPGGYG
Mouse EST VVVYTDGCCSSNGRKRARA
Human GIGVYWGPGHPLNVGI-RLPGRQTNQRAEIHAACKAIEQAKTQNIN-----KLVLYTDSMFTINGITNWVQGWKKN
Chicken GIGVYWGPGHPLNISE-RLPGRQTNQRAEIHAACKAIEQAKSQNIK-----KLIIYTDSKFTINGITSWVENWKTN
Yeast GYGAYFEGAPEENISEPLLSGAQTNNRAEIEAVSEALKKIWEKLTNEKEKVNYQIKTDSEYVTKLLNDRYMTYDNK
E.coli AILRYRGREKTFSAGY----TRTTNNRMELMAAIVALEALKEHC-------EVILSTDSQYVRQGITQWIHNWKKR
Mouse EST GIGVYWGPGHPLNVRI-RLPGRQTNQRAEIHAACKAVMQAKAQNIS-----KLVLYTDSMFTINGITNWVQGWKKN
Human GWKTSAGKEVINKEDFVALERL----------TQGMDIQWMHVPGHSGFIGNEEADRLAREGA-KQSED
Chicken GWRTSSGGSVINKEDFQKLDSL----------SKGIEIQWMHIPGHAGFQGNEEADRLAREGASKQKL
Yeast KLEGLPNSDLIVPLVQRFVKVKKYYELNKECFKNNGKFQIEWVKGHDGDPGNEMADFLAKKGASRR
E.coli GWKTADKKPVKNVDLWQRLDAA----------LGQHQIKWEWVKGHAGHPENERCDELARAAAMNPTLEDTGYQVEV
Mouse EST GWRTSTGKDVINKEDFMELDEL----------TQGMDIQWMHIPGHSGFVGNEE

Figure 1

Figure 2- RNase H2 Homology

```
                                          G DE GRGP  G  V A                                  DSK L
                                        p v * **a****vl*pm*y*                                *** *    SEQ ID NO:
Human:          MDLSELERDNTGRCRLSSPVPAVCRKE---PCVLGVDEAGRGPVLGPMVYAICYCPLPRLAD--LEALKVADSKTLL    6
Mouse:          MDLSELERDNTGRCRLSSPVPAVCLKE---PCVLGVDEAGRGPVLGPMVYAICYCPLSRLAD--LEALKVADSKTLT    7
C.elegans:   -SKTVKYFIERMSLKCETERSKTWNNFGNGI--PCVLGIDEAGRGPVLGPMVYAAAISPLDQNVE--LKNLGVDDSKALN    8
Yeast:        MVPPTVEASLESPYTKSYFSPVPSALLEQNDSPIIMGIDEAGRGPVLGPMVYAVAYSTQKYQDETIIPNYEFDDSKKLT    9
E.coli(H2):                              MIEFVYPHTQLVAGVDEVGRGPLVGAVVTAAVILDPARPIA------GLNDSKKLS   10

                 R                          P  I
                e  *  lf k   d              *  *s  m        y l    h     li                vdtvg
Human:          ESERERLFAKM-EDTDFVGWALDV---LSPNLISTSMLGRV---KYNLNSLSHDTATGLIQYALDQGVNVTQVFVDTVGM    6
Mouse:          ENERERLFAKMEEDGDFVGWALDV---LSPNLISTSMLGRV---KYNLNSLSHDTAAGLIQY?????????????????    7
C.elegans:      EAKREEIFNKMNEDEDIQQIIAYALRCLSPELISCSMLKRQ---KYSLNEVSHEAAITLIRDALACNVNVVEIKVDTVGP    8
Yeast:          DPIRRMLFSKIYQDNEELTQIGYATTCITPLDISRGMSKFPPTRNYNLNEQAHDVTMALIDGVIKQNVKLSHVYVDTVGP    9
E.coli(H2):     EKRRLALYEEIKEK--ALSWSLGR---AEPHEIDELNILHA---TMLAMQRAVAGLHIAPEYVLIDGNRC----------   10

                          P     V  K D      S AS  AKV RD
                   yq  l   f*g    *  *a* l p v*a**i *** **                          d   gsgyp dp t   l
Human:          PETYQARLQQSFPGIEVTVKAKADALYPVVSAASICAKVARDQAVKKWQFVEKLQ-DLDTDYGSGYPNDPKTKAWLKEHV    6
Mouse:          ????????????????????????????????????????????????????????????????????????????    7
C.elegans:      KATYQAKLEKLFPGISICVTEKADSLFPIVSAASIAAKVTRDSRLRNWQFREKNIKVPDAGYGSGYPGDPNTKKFLQLSV    8
Yeast:          PASYQKKLEQRFPGVKFTVAKKADSLYCMVSVASVVAKVTRDILVE------SLKRDPDEILGSGYPSDPKTVAWLKRNQ    9
E.coli(H2):     ---------PKLPMPAMAVV-KGDSRVPEISAASILAKVTRDAEMAALDIVFPQYGFAQHKGYPTAFHLEKLAEHGATEH   10

                    g      r sw t
Human:          EPVFGFPQ-FVRFSWRTAQTILEKEAEDVIWEDSASENQEGLRKITSYFLNEGSQARPRFSHRYFLERGLESATSL(end)    6
Mouse:          ????????????????????????????????????????????????????????????????????????????    7
C.elegans:      EPVFGFCS-LVRSSWKTASTIVEKRCVPGSWEDDEEEGKSQSKRMTSWMVPKNETEVVPKRNMEINLTKIVSTLFL-      8
Yeast:          TSLMGWPANMVRFSWQTCQTLLDDASKNSIPIKWEEQYMDSRKNAAQKTKQLQLQMVAKPVRRKRL-RTLDNWYR(end)    9
E.coli(H2):     HRRSFGPVKRALGLAS(end)                                                               10
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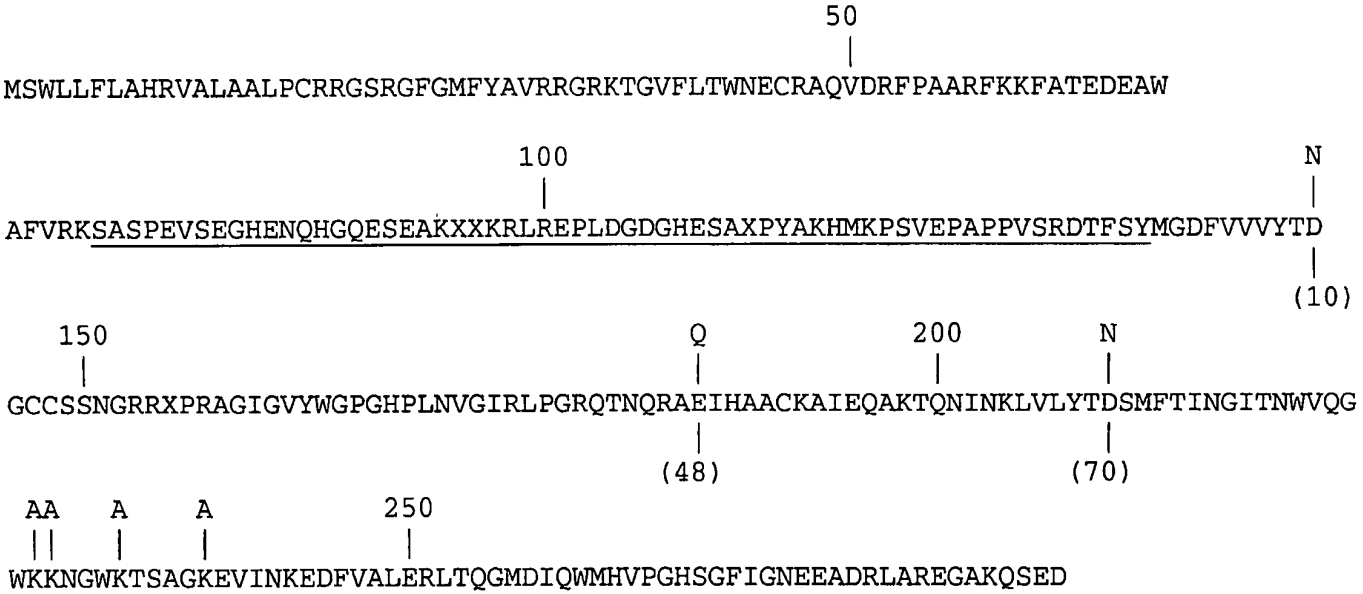

50
MSWLLFLAHRVALAALPCRRGSRGFGMFYAVRRGRKTGVFLTWNECRAQVDRFPAARFKKFATEDEAW
100
N
AFVRKSASPEVSEGHENQHGQESEAKXXKRLREPLDGDGHESAXPYAKHMKPSVEPAPPVSRDTFSYMGDFVVVYTD
(10)
150
Q
200
N
GCCSSNGRRXPRAGIGVYWGPGHPLNVGIRLPGRQTNQRAEIHAACKAIEQAKTQNINKLVLYTDSMFTINGITNWVQG
(48)
(70)
AA
A
A
250
WKKNGWKTSAGKEVINKEDFVALERLTQGMDIQWMHVPGHSGFIGNEEADRLAREGAKQSED

Figure 3A

Figure 3B

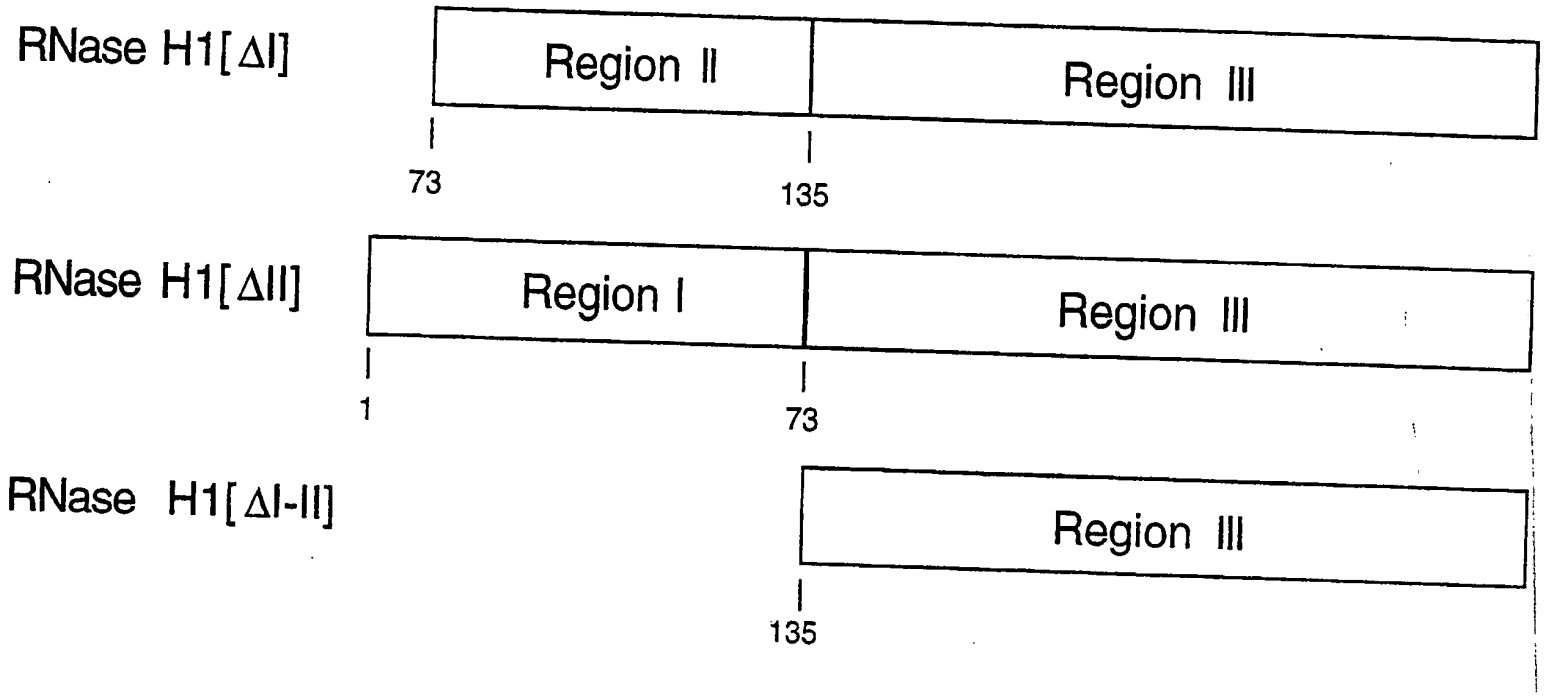
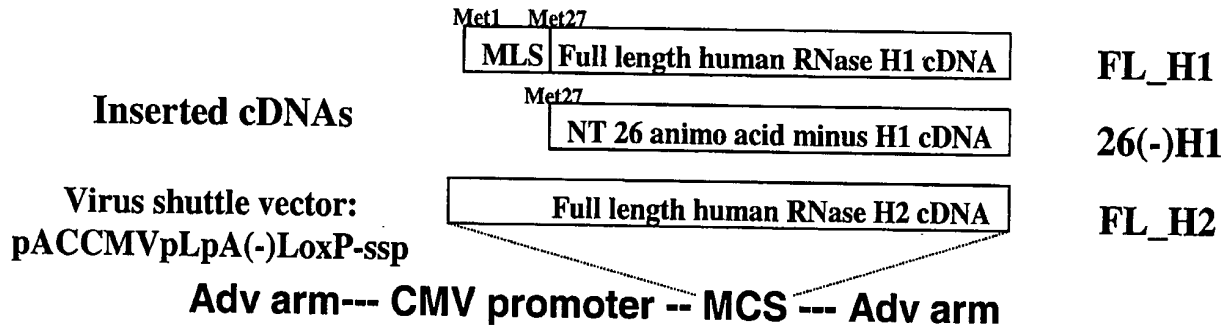

RNase H1[ΔI]
Region II
Region III
73
135
RNase H1[ΔII]
Region I
Region III
1
73
RNase H1[ΔI-II]
Region III
135

Figure 4 (A and B). Development of Adenoviruses (Adv) Over-expressing Human RNase H's

A.



B.

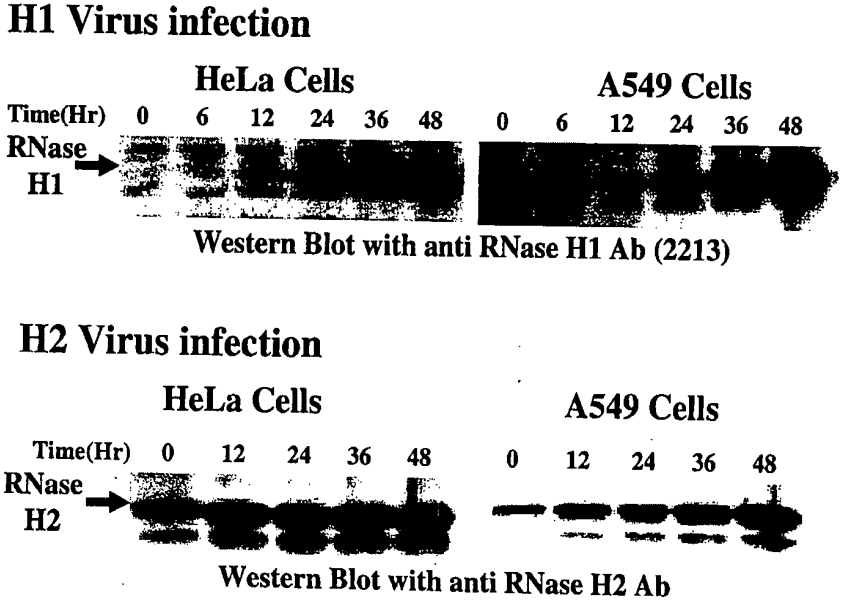

H1 Virus infection
HeLa Cells
A549 Cells
Time(Hr) 0 6 12 24 36 48 0 6 12 24 36 48
RNase H1
Western Blot with anti RNase H1 Ab (2213)
H2 Virus infection
HeLa Cells
A549 Cells
Time(Hr) 0 12 24 36 48 0 12 24 36 48
RNase H2
Western Blot with anti RNase H2 Ab

Figure 4 (C). Development of Adenoviruses (Adv) Over-expressing Human RNase H's (continued): Immunoprecipitation

C.

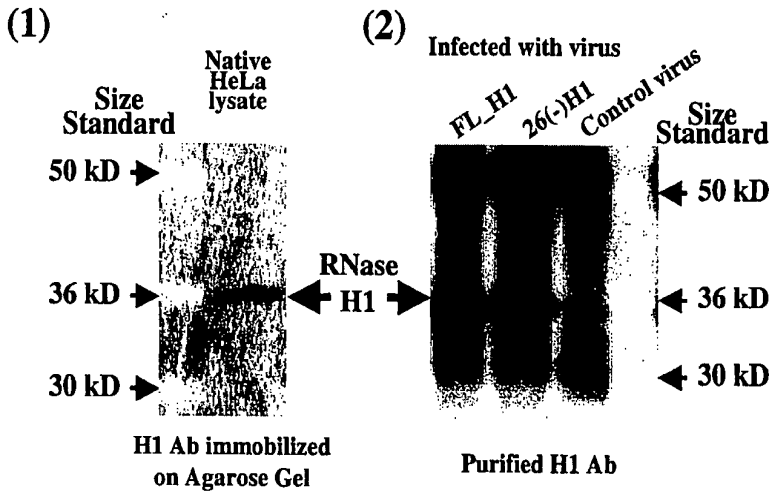


Figure 5 Development of the Adenovirus (Adv) Over-expressing Human RNase H's (continued): Gel renaturation Assay

E.

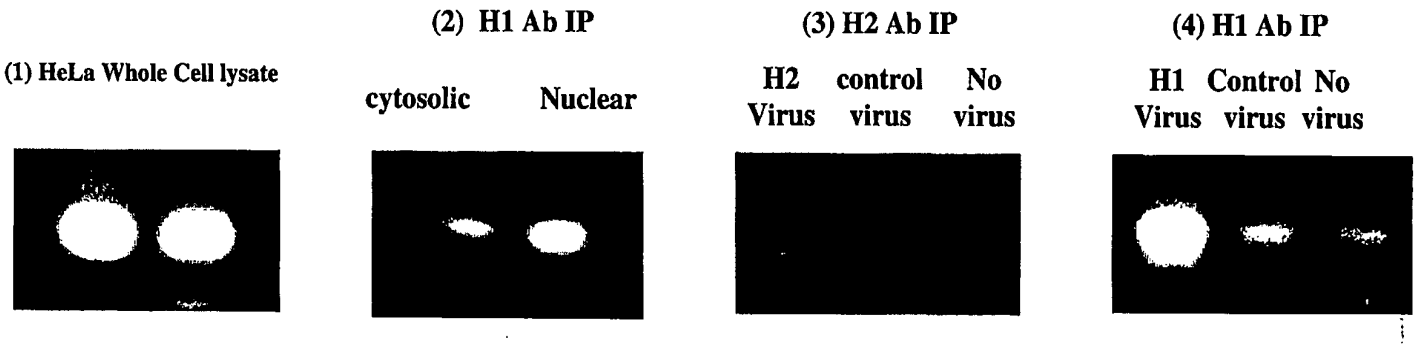


Figure 6. RNase H Ab IP Coupled TCA Assay

A.

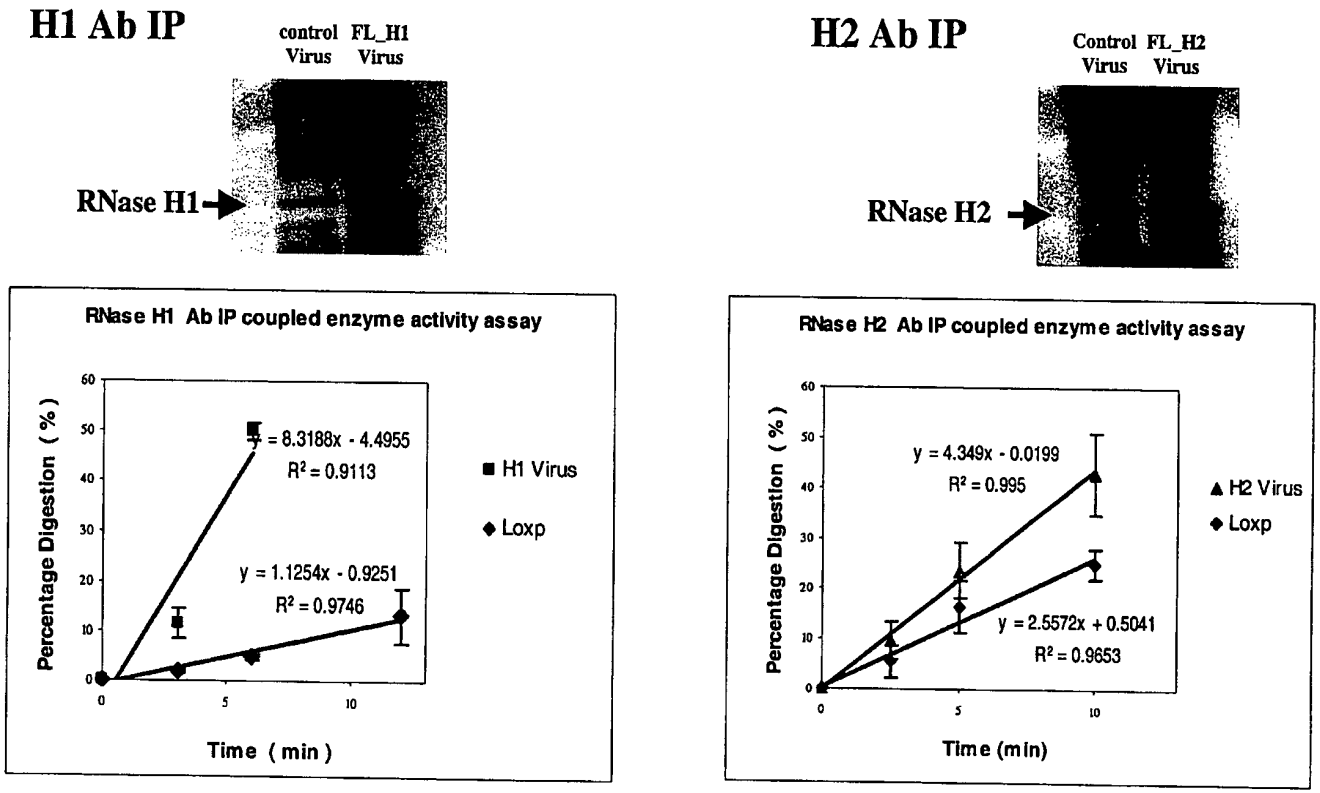
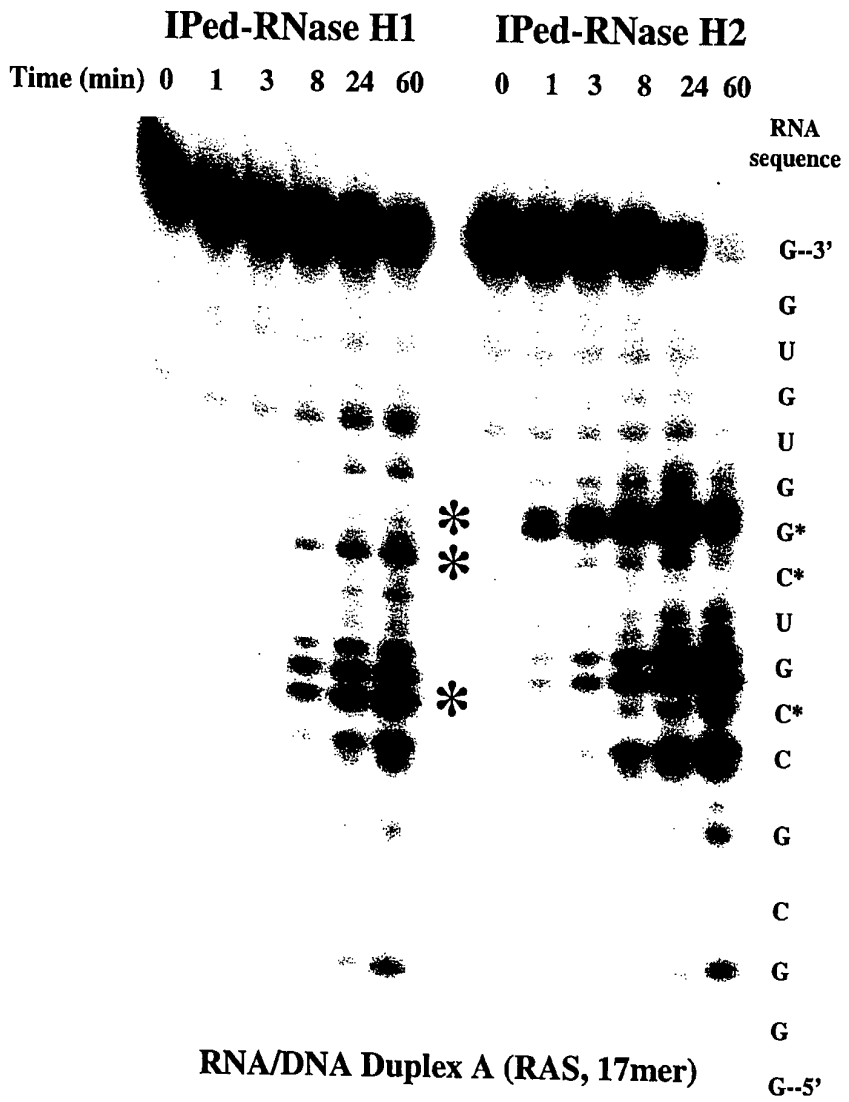


Figure 7A Different Digestion Patterns of Human RNase H1 and H2

A.



RNA/DNA Duplex A (RAS, 17mer)

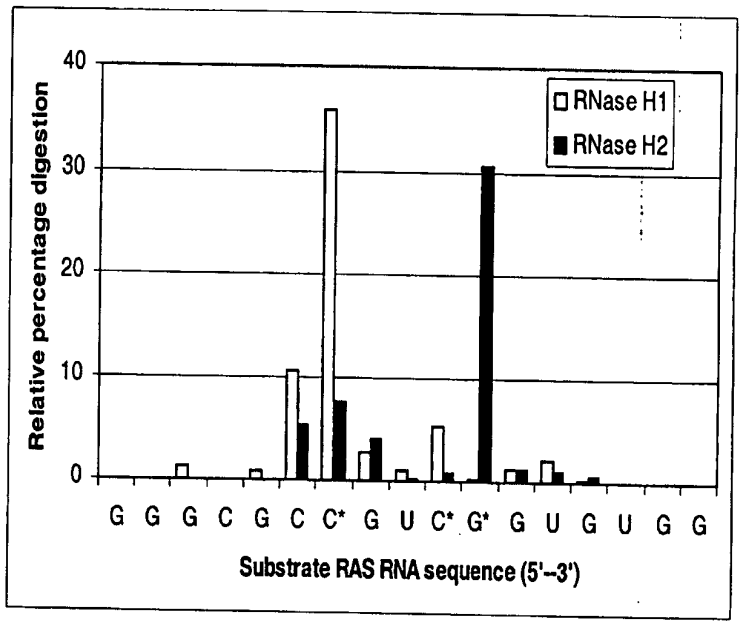
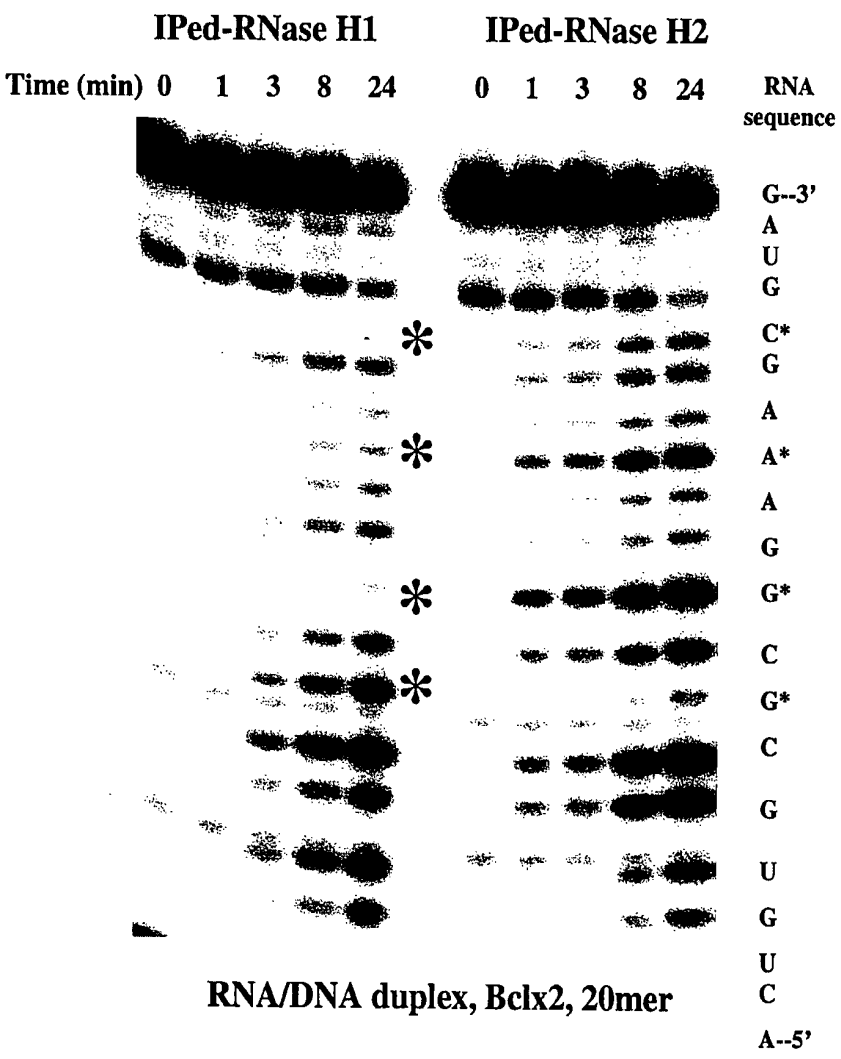


Figure 7B. Different Digestion Patterns of Human RNase H1 and H2

B.



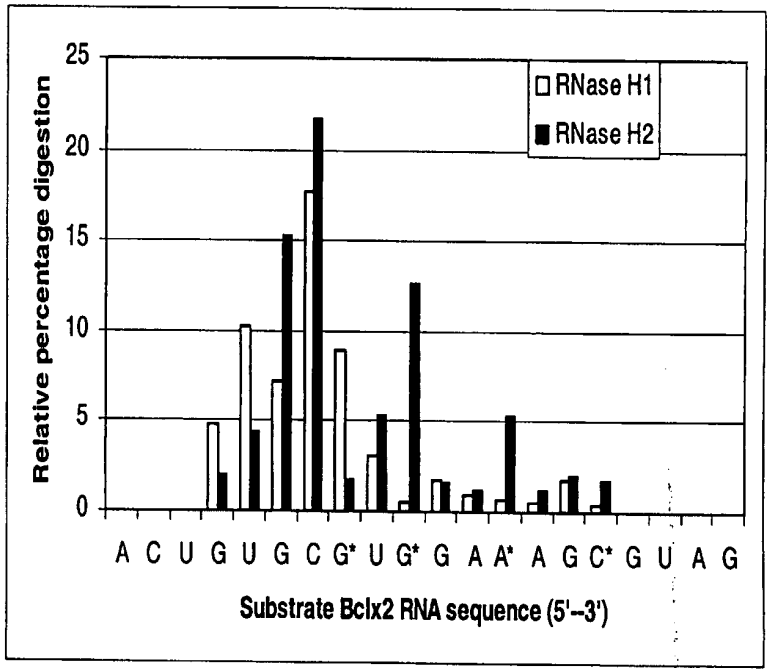
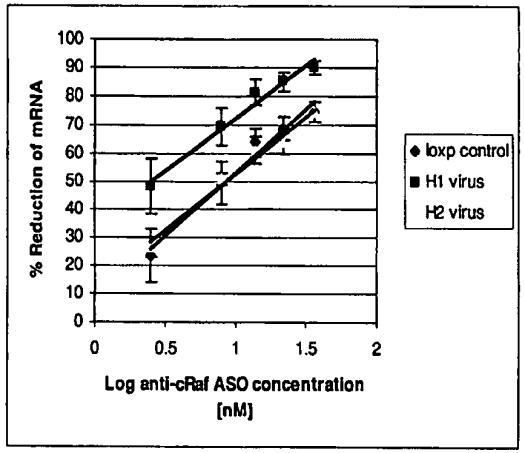


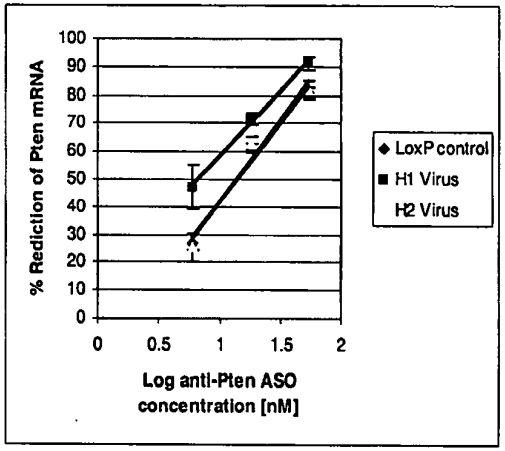
Figure 8. Effect of RNase H1 & H2 Overexpression on ASO (anti-cRaf, Pten and Jnk2) Potency in HeLa and A549 Cells: (A) Reduction of cRaf mRNA in HeLa cells treated with H1 or 2 virus; (B) Reduction of Pten mRNA in HeLa cells treated with H1 or 2 virus; (C) Reduction of Jnk2 mRNAin HeLa cells treated with H1 or 2 virus.

A.



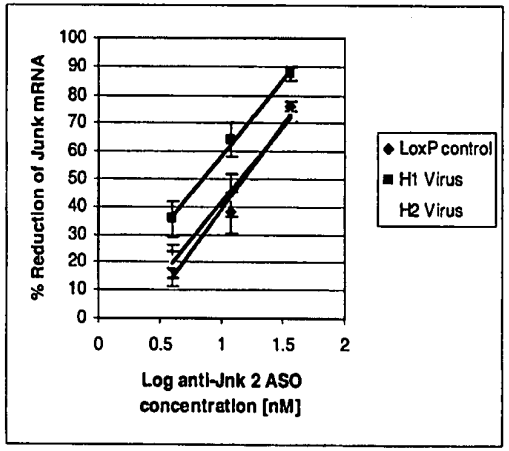
	Control	H1 Virus	H2 Virus
IC50 [nM]	8.65	2.59	8.65

B.



	Control	H1 Virus	H2 Virus
IC50 [nM]	14.06	6.61	13.90

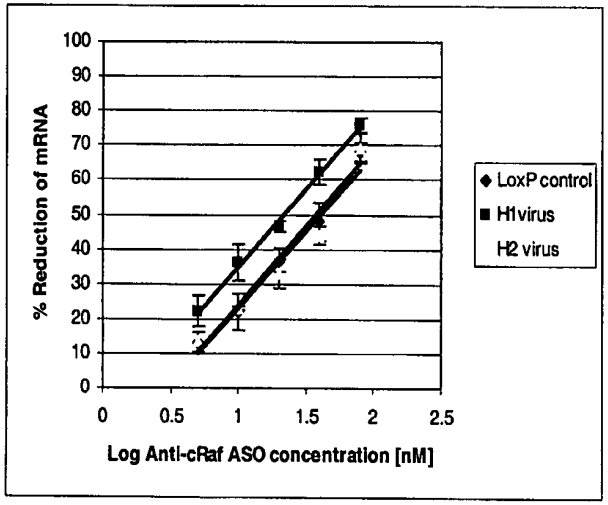
C.



	Control	H1 Virus	H2 Virus
IC50 [nM]	15.00	7.10	14.35

Figure 8. Effect of RNase H1 & H2 Overexpression on ASO (anti-cRaf, Pten and Junk2) Potency in HeLa and A549 Cells: (D) Reduction of cRaf mRNA in A549 cells treated with H1 or 2 virus; (E) Reduction of cRaf mRNA in HeLa cells treated with H1 virus

D.



	Control	H1 Virus	H2 Virus
IC_{50} [nM]	37.24	21.58	39.17

E.

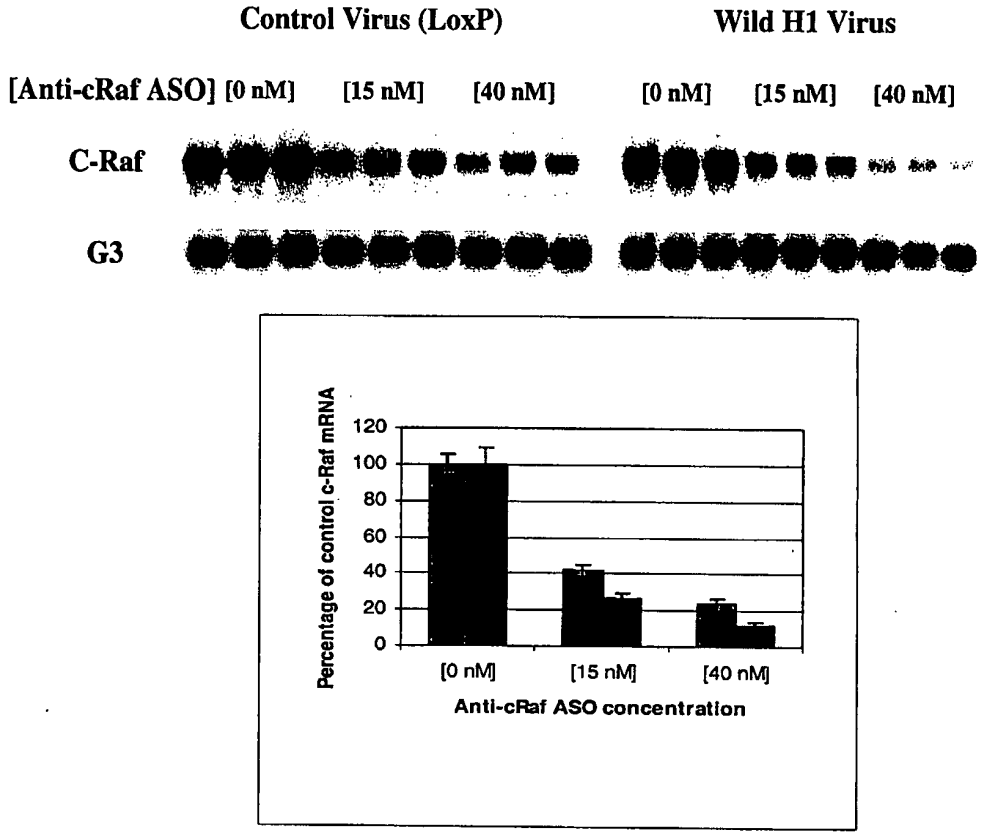
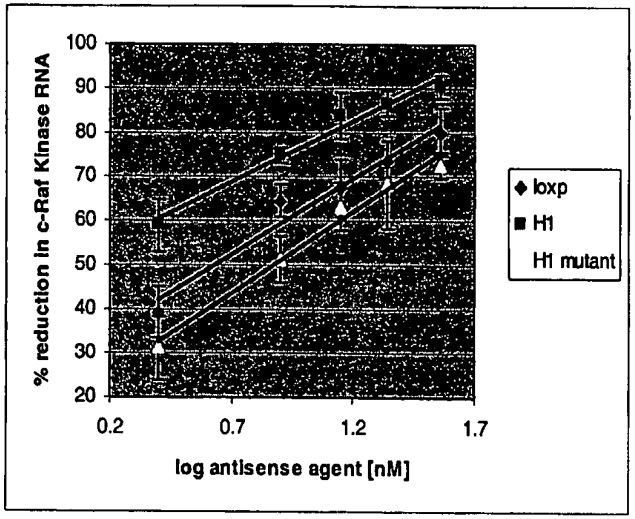


Figure 9. Effect Of RNase H1 & H2 And Mutant Overexpression On Antisense Drug Activity In HeLa Cells

A



B

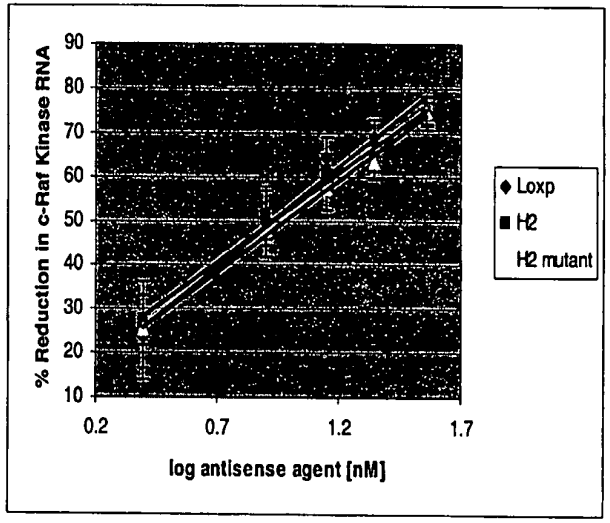
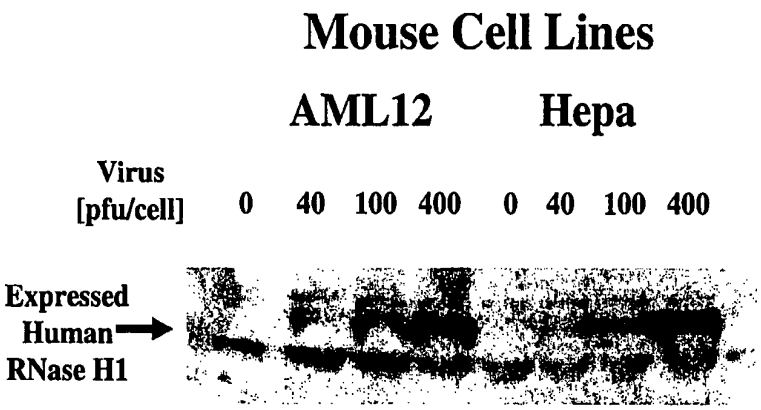


Figure 10. Overexpression of human RNase H1 enhances ASO potency in mouse cell line and mouse liver: (A) human RNase H1 expression in mouse cell lines; (B) Reduction of mouse Jnk 1 mRNA by ASO in Hepa cells infected with human RNase H1 or LoxP control viruses

A.



B.

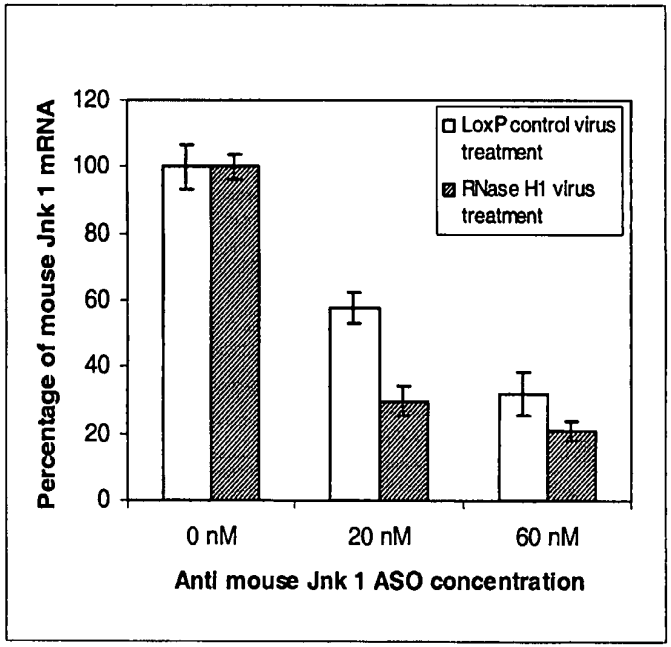
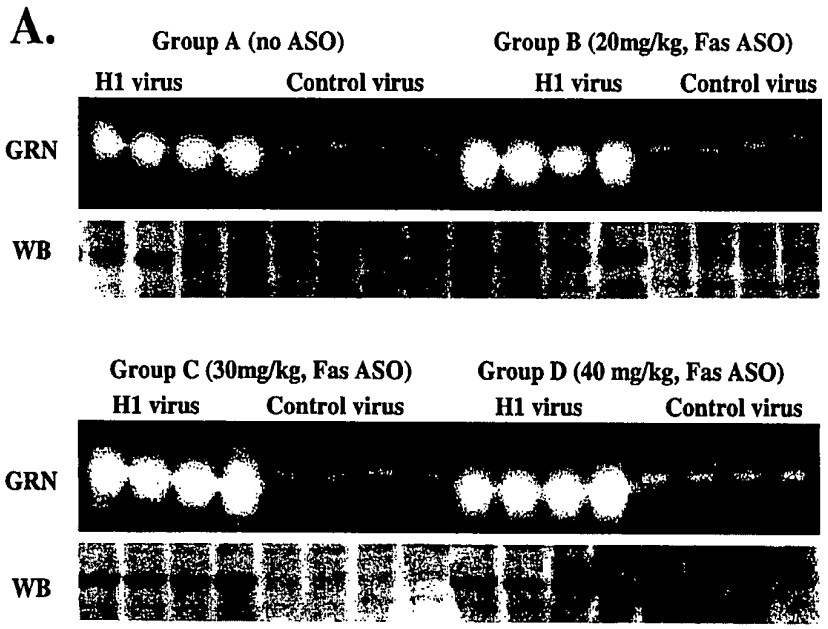
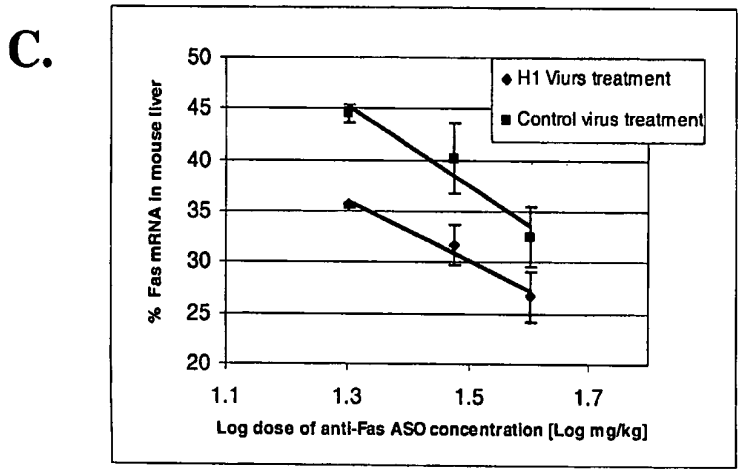


Figure 11. Overexpression of human RNase H1 enhances ASO potency in mouse liver: (A) Expression of human RNase H1 in mouse liver; (B) RNA protection assay; (C) Effects of Fas ASO on Fas mRNA levels in mouse liver.





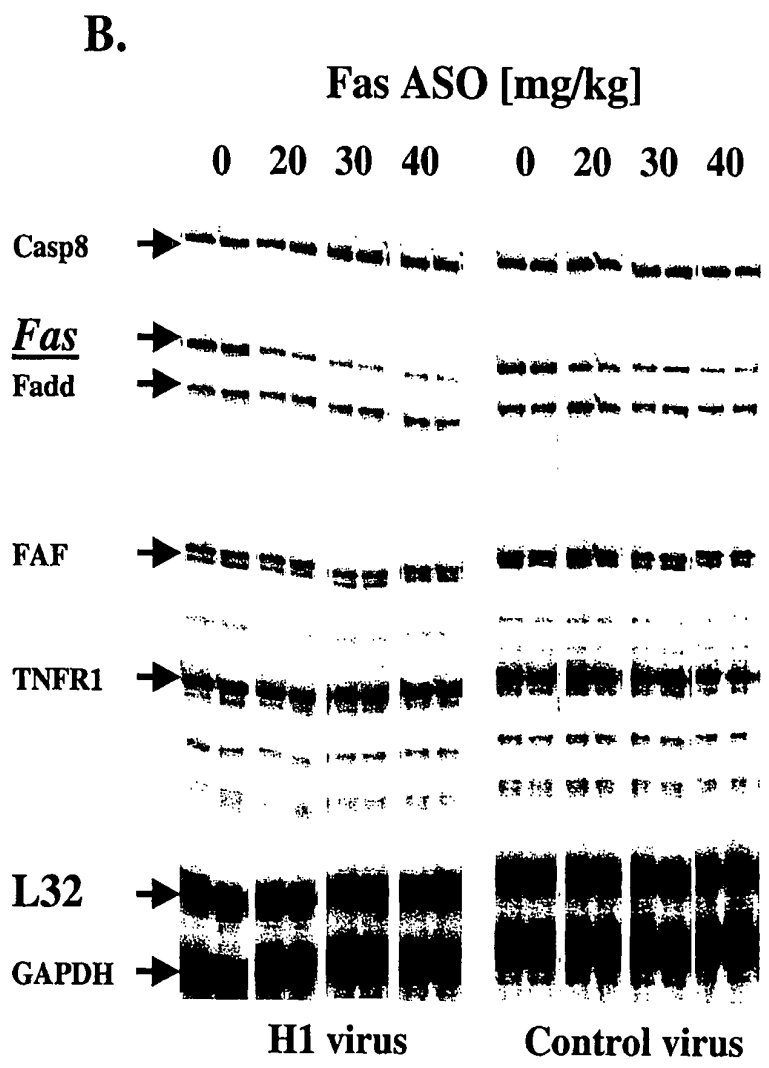


Figure 12. ASO 194178 and siRNA si-H1 inhibition of RNase H1 mRNA level and Enzyme Activity in HeLa and A549 Cells: (A) Northern Blot; (B) Gel renaturation assay.

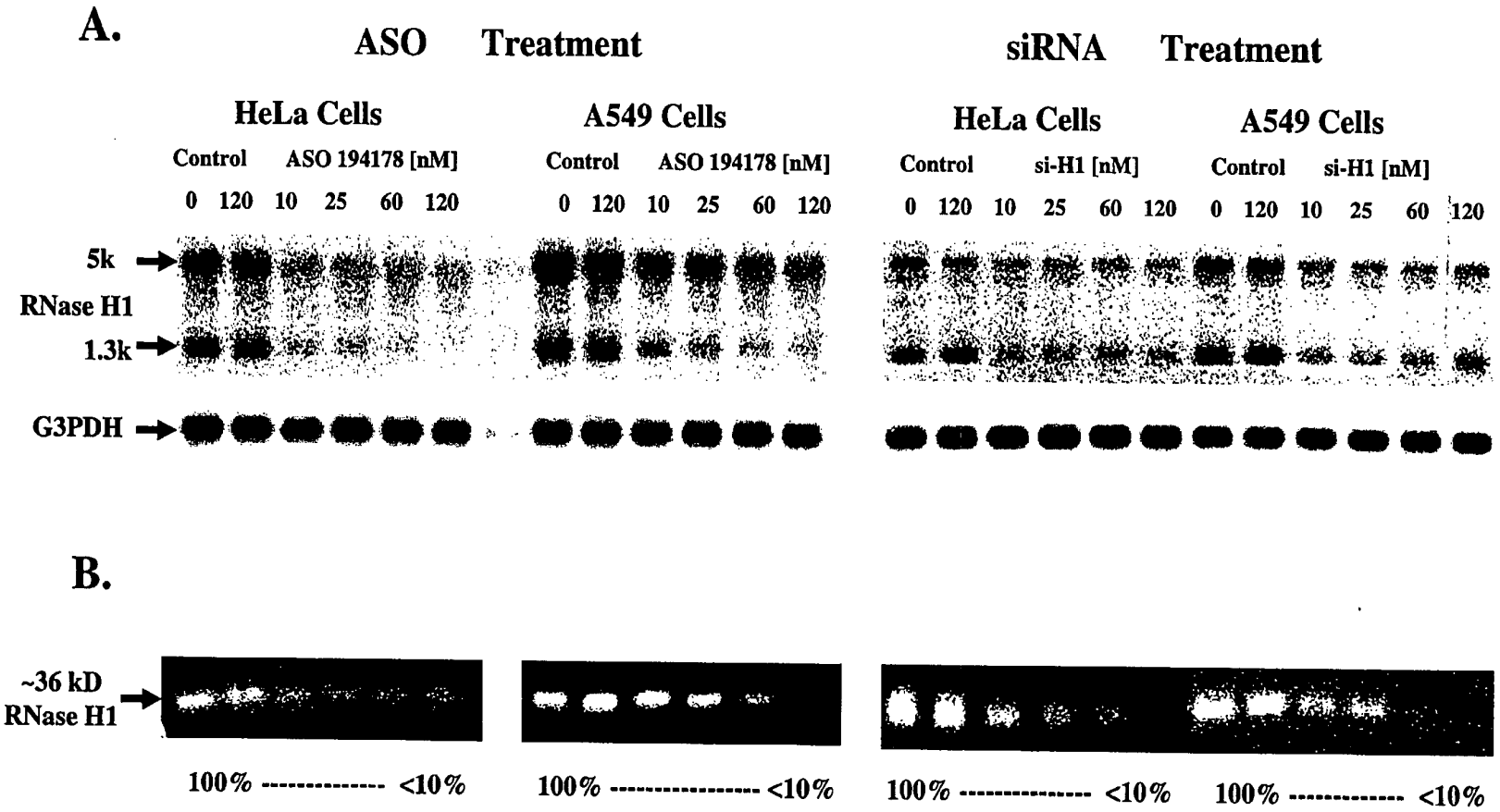


Figure 12. ASO 194186 and siRNA si21956 inhibition of RNase H2 mRNA and Protein level in HeLa and A549 Cells (continued): (C) Northern Blot; (D) Western Blot with anti H2 Ab.

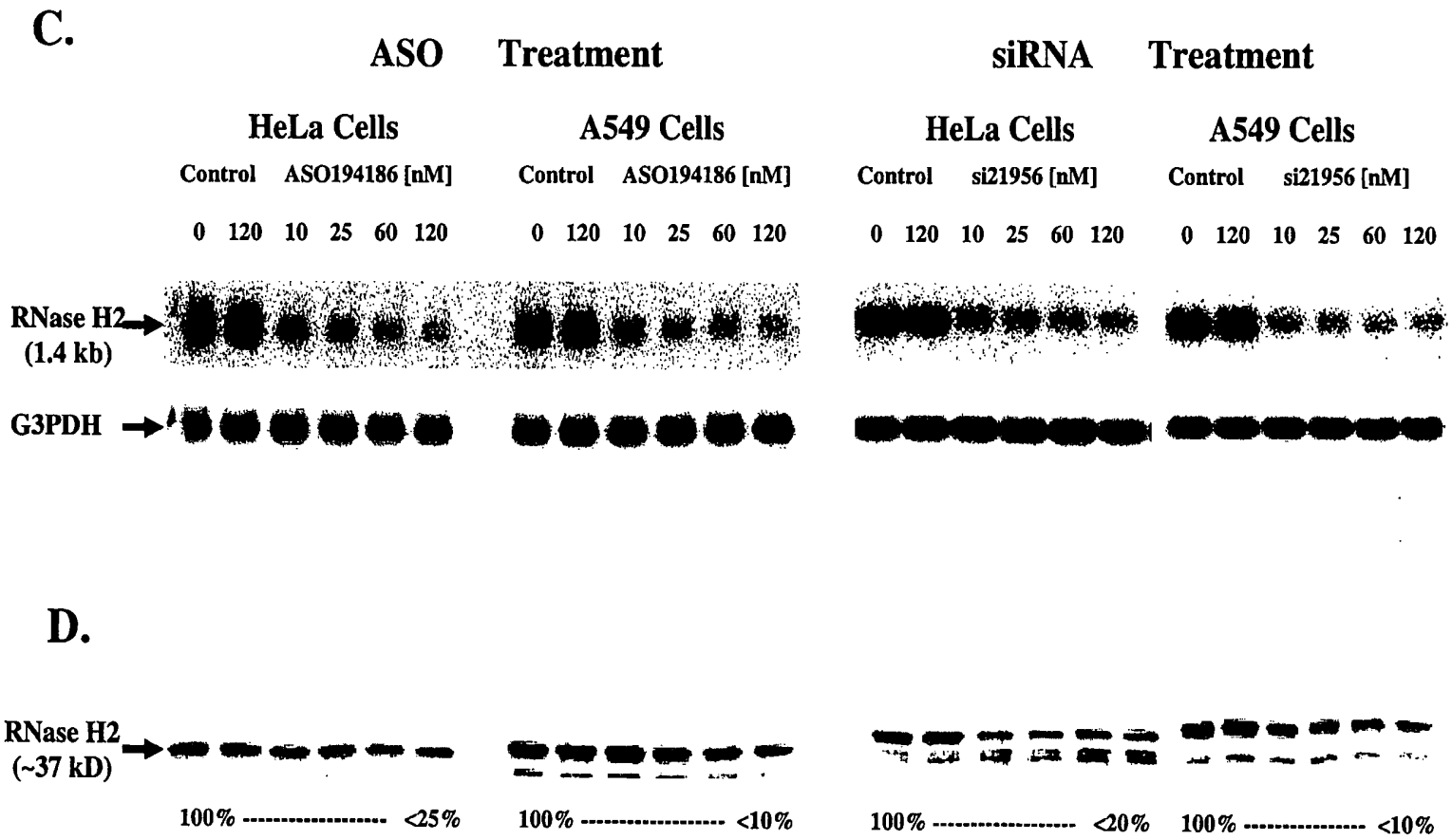
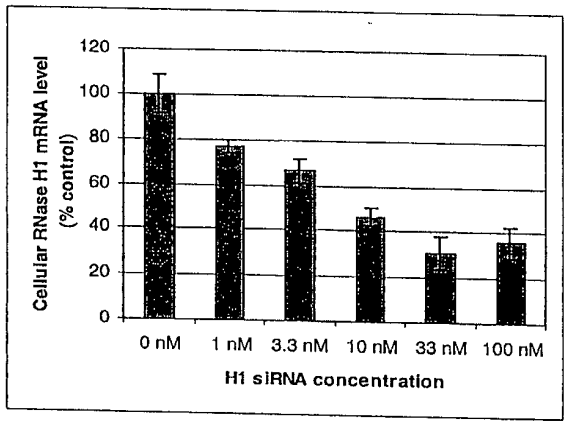
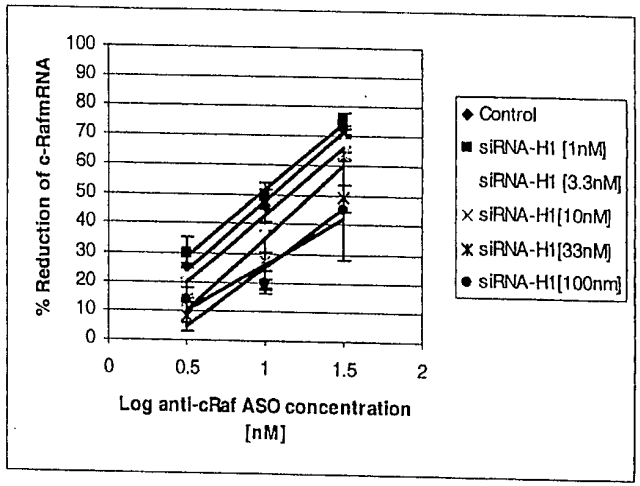


Figure 13 (A). Effects of RNase H1 &2 siRNA on ASO (ISIS 13650, c-Raf) Potency in HeLa and A549 Cells: (1) Cellular RNase H1 mRNA level; (2) Reduction of cRaf mRNA by ISIS 13650 in HeLa cells treated with H1 siRNA; (3) H1 mRNA lever correlates with ASO potency

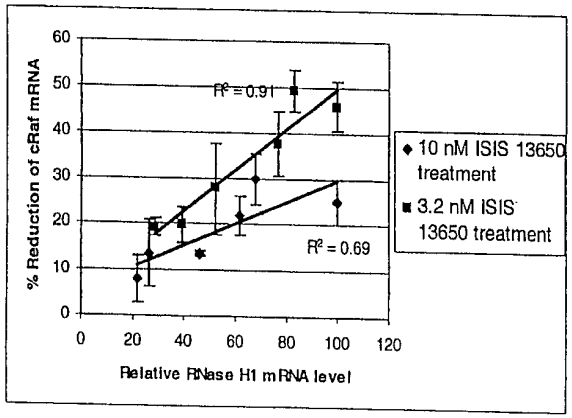
(1).



(2).



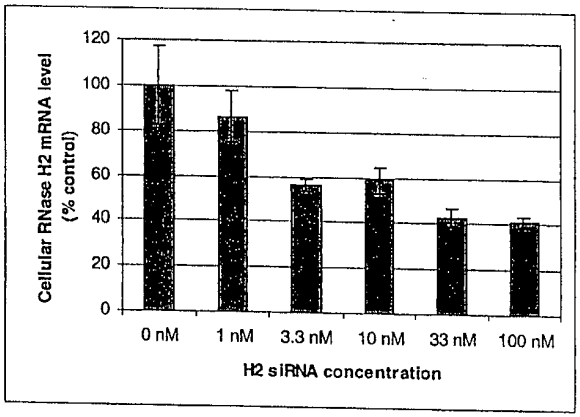
(3).



	IC_{50} [nM]
Control	11.25
siRNA-H1 [1nM]	9.38
siRNA-H1 [3.3nM]	14.45
siRNA-H1 [10nM]	19.59
siRNA-H1 [33nM]	37.15
siRNA-H1 [100nM]	47.75

Figure 13 (B). Effects of RNase H1 &2 siRNA on ASO (ISIS 13650, c-Raf) potency in HeLa and A549 Cells (continued): (1) Cellular RNase H2 mRNA level; (2) Reduction of cRaf mRNA by ISIS 13650 in HeLa cells treated with H2 siRNA; (3) H2 mRNA lever correlates with ASO potency

(1).



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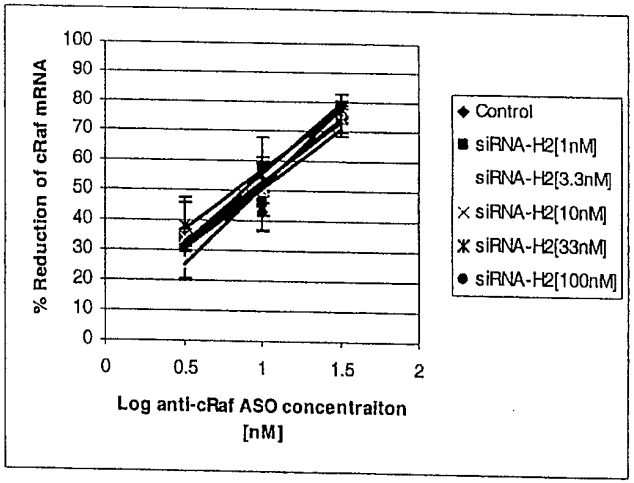

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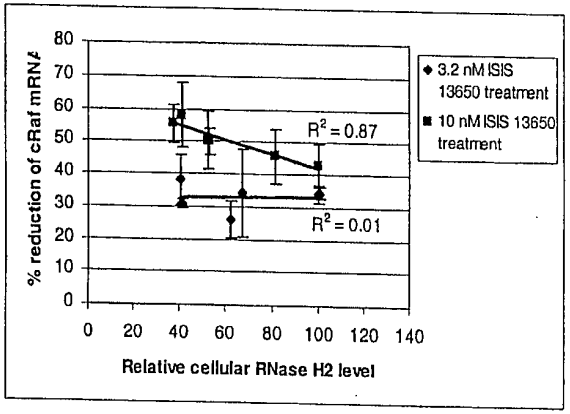
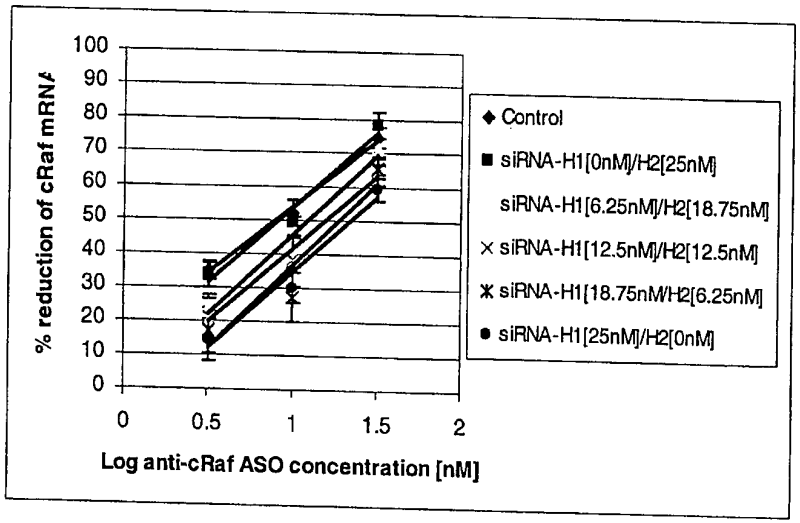


	IC_{50} [nM]
Control	9.89
siRNA-H2 [1nM]	8.87
siRNA-H2 [3.3nM]	9.42
siRNA-H2 [10nM]	8.63
siRNA-H2 [33nM]	6.71
siRNA-H2 [100nM]	7.67

Figure 13. Effects of RNase H1 &2 siRNA on ASO (ISIS 13650, c-Raf) Potency in HeLa and A549 Cells (continued): (C) Reduction of cRaf mRNA by ISIS 13650 in HeLa cells treated with H1 & H2 siRNA combination

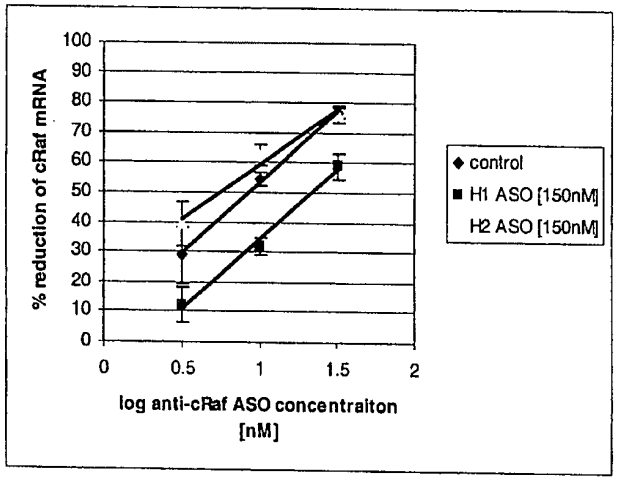
C.



	IC_{50} [nM]
Control	8.02
siRNA-H1[0 nM]/H2[25 nM]	8.30
siRNA-H1[6.25 nM]/H2[18.75 nM]	12.42
siRNA-H1[12.5 nM]/H2[12.5 nM]	15.74
siRNA-H1[18.75 nM]/H2[6.25 nM]	17.91
siRNA-H1[25 nM]/H2[0 nM]	21.48

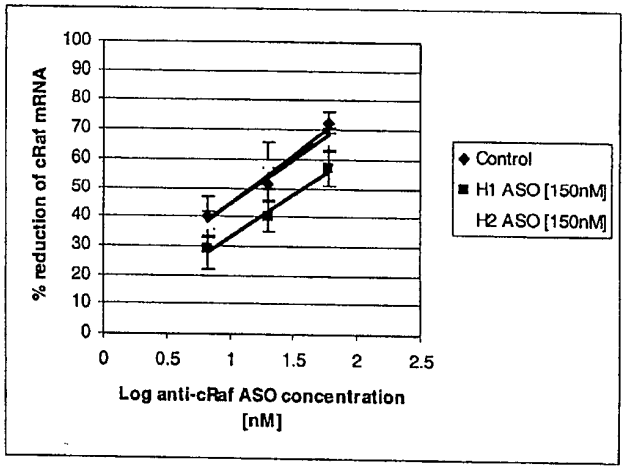
Figure 14. Effects of Antisense Reduction of RNase H1 & H2 on potency of c-Raf ASO (ISIS 13650) in HeLa and A549 Cells: (A) Reduction of cRaf mRNA by ISIS 13650 in HeLa cells treated with H1 or 2 ASO; (B) Reduction of cRaf mRNA by ISIS 13650 in A549 cells treated with H1 or 2 ASO.

A.



	Control	H1 ASO	H2 ASO
IC_{50} [nM]	8.61	21.73	5.71

B.



	Control	H1 ASO	H2 ASO
IC_{50} [nM]	14.62	36.73	15.17

Figure 15. Several RNase H's are present in human cells: (A) Gel renaturation assay in the presence of Mg++; (B) Gel renaturation in the presence of Mn++

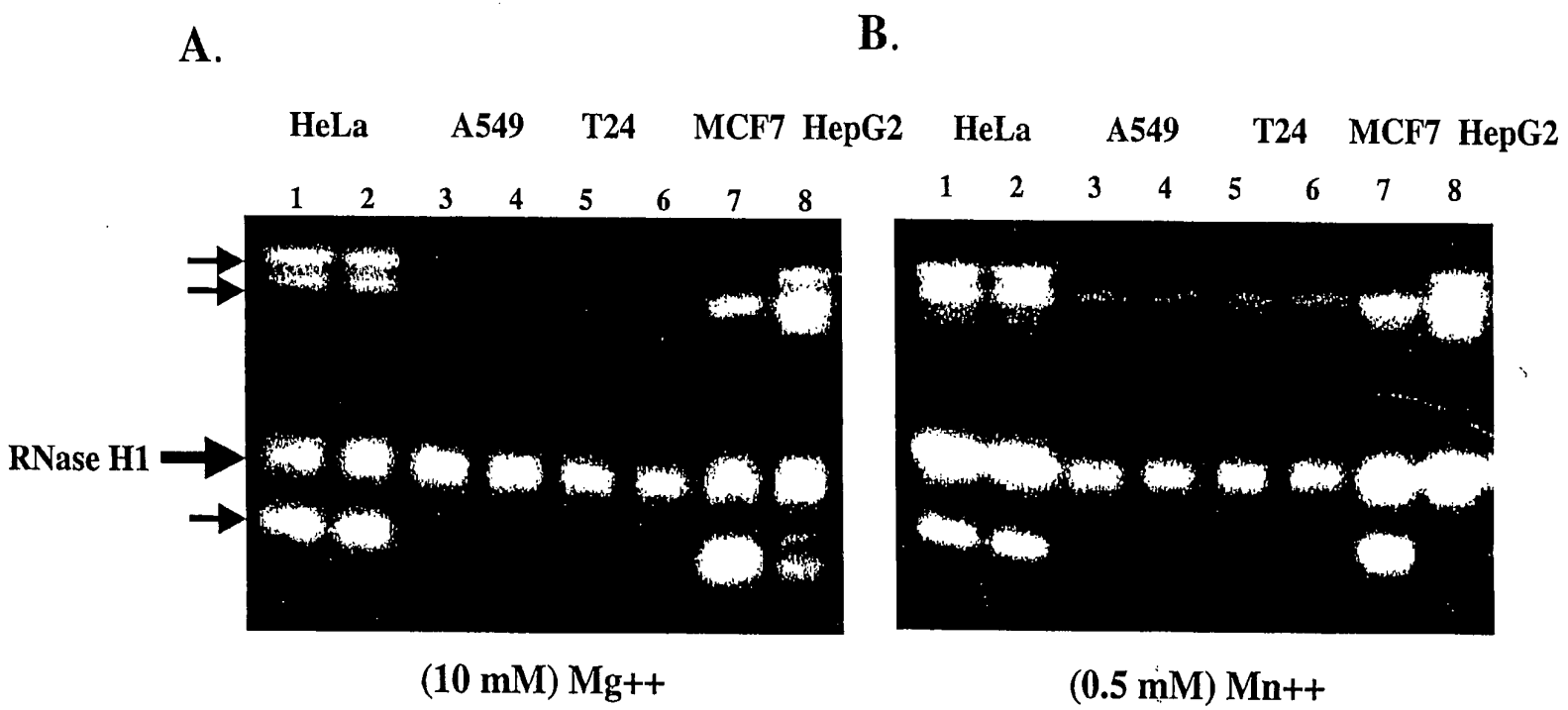
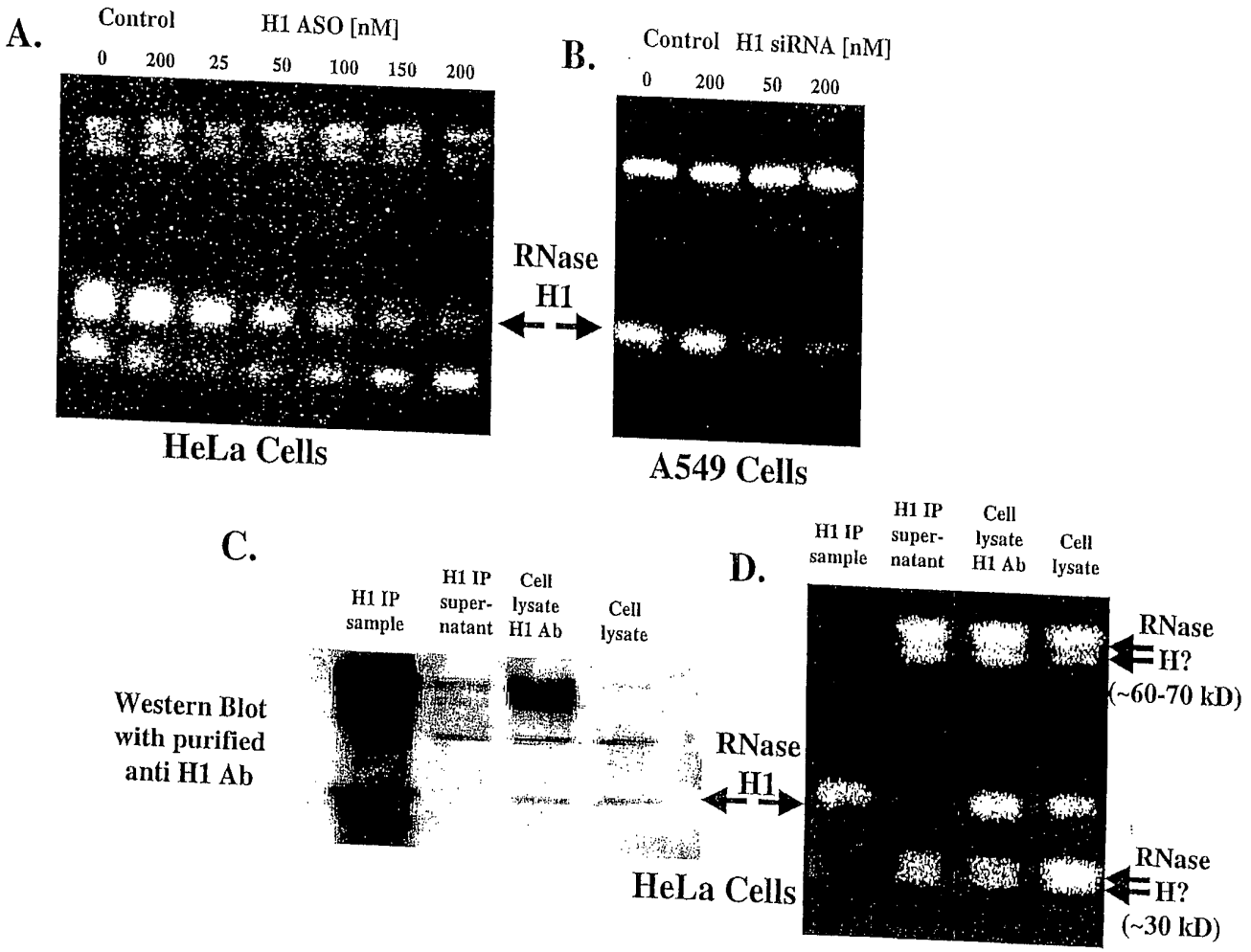



Figure 16. There Are several human RNase H activities other than H1 and H2: (A) Gel renaturation assay of HeLa cell lysate pretreated with RNase H1 ASO (ISIS194178); (B) Gel renaturation assay of A549 cell pretreated with RNase H1 siRNA; (C and D) Western Blot analysis and gel renaturation assay of HeLa cell lysate before and after immunoprecipitation with RNase H1 Ab